

## [CLAIMS]

- a.
- Sub Bi
1. An *in vitro* assay method which comprises:
- 5 a) using an assay reagent containing at least one NMR active nucleus to perform an assay, and
- b) hyperpolarising at least one NMR active nucleus of the assay reagent;
- wherein steps (a) and (b) are performed simultaneously or sequentially in either order, and
- c) analysing the assay reagent and/or the assay by NMR and
- 10 d) optionally using the NMR data obtained in step c) to generate further assay result(s).
2. The method of claim 1 wherein the NMR active nucleus is  $^{15}\text{N}$ ,  $^{19}\text{F}$ ,  $^{31}\text{P}$ ,  $^1\text{H}$ ,  $^{29}\text{Si}$  and/or  $^{13}\text{C}$ .
3. The method of claim 2, wherein the NMR active nucleus is  $^{15}\text{N}$  or  $^{13}\text{C}$ .
4. The method of any of claims 1 to 3, wherein the assay reagent is a compound which contains an artificially high concentration of an NMR active nucleus.
5. The method of claim 4, wherein the assay reagent contains an artificially high concentration in 1-10 defined positions.
- 25 6. The method of any of claims 1 to 5, wherein the assay reagent is an organic compound comprising one or more NMR active nuclei associated with a bond which is broken during the course of the assay.
- 30 7. The method of claim 6, wherein the assay reagent contains two or more NMR active nuclei and each NMR active nucleus produces a distinct NMR spectrum and when the
- Sub a-1

Sub B1  
assay method is performed, it results in changes to the chemical and/or physical environment of the nucleus and this is mirrored by spectral changes which can be monitored.

Subs. Q3  
The method of any claim 1-7, wherein the assay reagent is analysed repeatedly in step c) at known time intervals so as to generate information about a change with time of the assay reagent.

Sub Q4  
The method of any one claim 1 to 8, wherein the assay reagent is a Nucleotide, or nucleotide analogue, polynucleotide, amino acid analogue, polypeptide or protein.

Sub Q5  
The method of any one of claims 1 to 9, wherein the assay is a nucleic acid hybridisation assay.

Sub Q6  
The method of any one of claims 1 to 10, wherein the assay is a binding assay.

Sub Q7  
The method of claims 1 to 11, wherein the assay reagent is a compound specifically labelled with at least one NMR active nucleus and the assay reagent is administered to a micro-organism, macro-organism or cultured cells, cellular metabolites or an excretion product of the assay reagent are hyperpolarised and analysed by nuclear magnetic resonance spectroscopy, nuclear magnetic resonance imaging or both.

Sub Q8  
The method of claims 1 to 12, wherein the assay is a binding study performed using micro-organisms or cultured cells.

Sub Q9  
The method of claims 1 to 13 wherein the hyperpolarisation transfer is repeated to enhance the signal-to-noise ratio.

Sub Q10  
The method of claim 1 to 14 wherein the shortening effect as expressed by the improvement of signal-to-noise per unit time is a factor of 10 or more compared to known assay techniques without hyperpolarisation.

Sub 10  
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b1  
The method of claims 1 to 15 where the hyperpolarisation of the NMR active nucleus of the assay reagent is carried out by polarisation transfer from a hyperpolarised noble gas, or a mixture of hyperpolarised noble gases.

5 17. The method of claim 16 wherein the noble gas is  $^{129}\text{Xe}$ .

18. The method of claim 16 wherein the noble gas is  $^3\text{He}$ .

Sub 19  
a 12  
The method of claims 16 to 18 wherein the hyperpolarisation is transferred by a hyperpolarised noble gas in solution and wherein the viscosity of the solution is at least 1000 mPs.

Sub 20  
a 13  
The method of claims 1 to 15 where the hyperpolarisation of the NMR active nucleus of the assay reagent is carried out by polarisation transfer at a temperature of 4.2 K or less in the presence of a magnetic field of at least 1 T.

Sub 21  
a 14  
The method of claims 1 to 15 where the hyperpolarisation of the NMR active nucleus of the assay reagent is carried out by polarisation transfer using dynamic nuclear polarisation.

Sub 22  
a 15  
The method of claims 1 to 15 where the hyperpolarisation of the NMR active nucleus of the assay reagent is carried out by para hydrogen induced polarisation.

Sub 23  
a 16  
The method of claims 1 to 15 where the hyperpolarisation of the NMR active nucleus of the assay reagent is carried out with the spin refrigeration technique.

Sub 24  
a 17  
The method of claims 1 to 23, wherein more than one assay is multiplexed and monitored by NMR spectroscopy and/or NMR imaging.

Sub 25  
a 18  
The method of claims 1 to 24 wherein the assay is performed in a multiwell or multispot assay array.

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26. The method of claims 1 to 25 wherein step c) is performed by examining the assay reagent using both NMR spectroscopy to obtain more than one spectrum, and magnetic resonance imaging to obtain one or more discrete spectral location, and repeating the examination at least once so as to obtain quantitative information about kinetic or time-dependant alteration in chemistry, environment or structure of the assay reagent.

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A 20

27. The method of claim 1 to 26, wherein step c) is performed in an aerosol or flow-through device applied to aerosol droplets where the well, surface or container is used to contain the assay reagent.

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A 21

28. An *in vitro* assay kit for carrying out the assay method as defined in claim 1 to 27 which comprises: one or more assay reagents each containing at least one NMR active nucleus contained in a well or vial or other suitable container for carrying out the hyperpolarisation of step (b) of claim 1.

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A 22

29. The *in vitro* kit of claim 28 where the NMR analysis of step (c) of claim 1 is carried out in the same well, vial or container as the hyperpolarisation transfer is carried out.